10

15

20

#### What is claimed is:

- An isolated nucleic acid comprising:
  a nucleotide sequence depicted in SEQ ID NO: 1 or SEQ ID NO: 3.
- 2. A polypeptide encoded by a nucleotide sequence according to claim 1.
- 3. A purified or isolated polypeptide comprising an amino acid sequence depicted in SEQ ID NO: 2 or SEQ ID NO: 4.
- 4. An isolated nucleic acid encoding a polypeptide having an amino acid sequence depicted in SEQ ID NO: 2 or SEQ ID NO: 4.
- 5. An isolated nucleic acid comprising a nucleotide sequence which encodes a polypeptide which desaturates a fatty acid molecule at carbon 6 or 12 from the carboxyl end of said polypeptide, wherein said nucleotide sequence has an average A/T content of less than about 60%.
- 6. The isolated nucleic acid according to Claim 5, wherein said nucleic acid is derived from a fungus.
  - 7. The isolated nucleic acid according to Claim 6, wherein said fungus is of the genus Mortierella.
- 25 8. The isolated nucleic acid according to Claim 7, wherein said fungus is of the species Mortierella alpina.

5

10

- An isolated nucleic acid, wherein the nucleotide sequence of said nucleic 9. acid is depicted in SEQ ID NO: 1. or SEQ ID NO: 3.
- An isolated or purified polypeptide which desaturates a fatty acid molecule at 10. carbon 6 or 12 from the carboxyl end of said polypeptide, wherein said polypeptide is a eukaryotic polypeptide or is derived from a eukaryotic polypeptide.
- The isolated or purified eukaryotic polypeptide according to Claim 10, 11. wherein said eukaryotic polypeptide is derived from a fungus.
- 12. A nucleic acid comprising!

a fungal nucleotide sequence which is substantially identical to a sequence of at least 50 nucleotides in SEQ ID NO: \ or SEQ ID NO: 3 or is complementary to a sequence of at least 50 nucleotides in SEQ ID NO: 1 or SEQ ID NO: 3.

- An isolated nucleic acid having a nucleotide sequence with at least about 13. 50% homology to SEQ ID NO: 1 or SEQ NO: 3.
- 14. An isolated nucleic acid having a nucleotide sequence with at least about 50% homology to sequence encoding an amino\acid sequence depicted in SEQ ID 20 NO: 2 or SEQ ID NO: 4.
  - The nucleic acid of claim 14, wherein said amino acid sequence depicted in 15. SEQ ID NO: 2 is selected from the group consisting of amino acid residues 50-53, 39-43, 172-176, 204-213, and 390-402.
    - A nucleic acid construct comprising: 16.

15

20

25

a nucleotide sequence depicted in a SEQ ID NO: 1 or SEQ ID NO: 3 linked to a heterologous nucleic acid.

- 17. A nucleic acid construct comprising:
- a nucleotide sequence depicted in a SEQ ID NO: 1 or SEQ ID NO: 3 operably associated with an expression control sequence functional in a microbial cell.
  - 18. The nucleic acid construct according to Claim 17, wherein said microbial cell is a yeast cell.
  - 19. The nucleic acid construct according to Claim 17, wherein said nucleotide sequence is derived from a fungus.
  - 20. The nucleic acid construct according to Claim 19, wherein said fungus is of the genus Mortierella.
  - 21. The nucleic acid construct according to Claim 20, wherein said fungus is of the species *Mortierella alpina*.
  - 22. A nucleic acid construct comprising:
  - a fungal nucleotide sequence which encodes a polypeptide comprising an amino acid sequence which corresponds to or is complementary to an amino acid sequence depicted in SEQ ID NO: 2 or SEQ ID NO: 4, wherein said nucleic acid is operably associated with an expression control sequence functional in a microbial cell, wherein said nucleotide sequence encodes a functionally active polypeptide which desaturates a fatty acid molecule at carbon 6 or 12 from the carboxyl end of a fatty acid molecule.

10

15

20

25

#### 23. A nucleic acid construct comprising:

a nucleotide sequence having an A/T content of less than about 60% which encodes a functionally active  $\Delta 6$ -desaturase having an amino acid sequence which corresponds to or is complementary to all of or a portion of an amino acid sequence depicted in a SEQ ID NO: 2, wherein said nucleotide sequence is operably associated with a transcription control sequence functional in a yeast cell.

#### 24. A nucleic acid construct comprising:

a fungal nucleotide sequence which encodes a functionally active  $\Delta 12$ -desaturase having an amino acid sequence which corresponds to or is complementary to all of or a portion of an amino acid sequence depicted in a SEQ ID NO: 4, wherein said nucleotide sequence is operably associated with a transcription control sequence functional in a yeast cell.

25. A recombinant yeast cell comprising:

a nucleic acid construct according to Claim 23 or Claim 24.

26. The recombinant yeast cell according to Claim 25, wherein said yeast cell is a Saccharomyces cell.

## 27. A recombinant yeast cell comprising:

at least one copy of a vector comprising a fungal nucleotide sequence which encodes a polypeptide which converts 18:2 fatty acids to 18:3 fatty acids or 18:3 fatty acids to 18:4 fatty acids, wherein said yeast cell or an ancestor of said yeast cell was transformed with said vector to produce said recombinant yeast cell, and wherein said nucleotide sequence is operably associated with an expression control sequence functional in said recombinant yeast cell.

WO 98/46763 PCT/US98/07126

28. The recombinant yeast cell according to claim 27, wherein said fungal nucleotide sequence is a *Mortierella* nucleotide sequence.

5

- 29. The recombinant yeast cell according to Claim 28, wherein said recombinant yeast cell is a Saccharomyces cell.
- 30. The microbial cell according to Claim 27, wherein said expression control sequence is provided in said expression vector.

10 1

Ĺ

15

31. A method for production of GLA in a yeast culture, said method comprising:

growing a yeast culture having a plurality of recombinant yeast cells, wherein said yeast cells or an ancestor of said yeast cells were transformed with a vector comprising fungal DNA encoding a polypeptide which converts LA to GLA, wherein said DNA is operably associated with an expression control sequence functional in said yeast cells, under conditions whereby said DNA is expressed, whereby GLA is produced from LA in said yeast culture.

20

- 32. The method according to Glaim 31, wherein said fungal DNA is Mortierella DNA and said polypeptide is a  $\Delta 6$  desaturase.
- 33. The method according to Claim 32, wherein Mortierella is of the species Mortierella alpina.

25

34. The method according to Claim 31, wherein said LA is exogenously supplied.

10

15

20

25

- 35. The method according to Claim 31, wherein said conditions are inducible.
- 36. A method for production of stearidonic acid in a yeast culture, said method comprising:

growing a yeast culture having a plurality of recombinant yeast cells, wherein said yeast cells or an ancestor of said yeast cells were transformed with a vector comprising fungal DNA encoding a polypeptide which converts  $\alpha$ -linolenic acid to stearidonic acid, wherein said DNA is operably associated with an expression control sequence functional in said yeast cells, under conditions whereby said DNA is expressed, whereby stearidonic acid is produced from  $\alpha$ -linolenic acid in said yeast culture.

- 37. The method according to Claim 36, wherein said fungal DNA is Mortierella DNA and said polypeptide is a Δ6 desaturase.
- 38. The method according to claim 37, wherein Mortierella is of the species Mortierella alpina.
- 39. The method according to Claim 36, wherein said α-linolenic acid is exogenously supplied.
  - 40. The method according to Claim 36, wherein said conditions are inducible.
  - 41. A method for production of linoleic acid in a yeast culture, said method comprising:

10

15

20

growing a yeast culture having a plurality of recombinant yeast cells, wherein said yeast cells or an ancestor of said yeast cells were transformed with a vector comprising fungal DNA encoding a polypeptide which converts oleic acid to linoleic acid, wherein said DNA is operably associated with an expression control sequence functional in said yeast cells, under conditions whereby said DNA is expressed, whereby linoleic acid is produced from oleic acid in said yeast culture.

- 42. The method according to Claim 41, wherein said fungal DNA is Mortierella DNA and said polypeptide is a Δ12 desaturase.
- 43. The method according to Claim 42, wherein *Mortierella* is of the species *Mortierella alpina*.
- 44. The method according to Claim 41, wherein said conditions are inducible.
- 45. An isolated or purified polypeptide which desaturates a fatty acid molecule at carbon 12 from the carboxyl end of said polypeptide, wherein said polypeptide is a fungal polypeptide or is derived from a fungal polypeptide.
- 46. The isolated or purified polypeptide according to Claim 46, wherein said polypeptide is a *Mortierrella alpina* Δ12 desaturase.
- 47. An isolated or purified polypeptide which desaturates a fatty acid molecule at carbon 6 from the carboxyl end of said polypeptide, wherein said polypeptide is a fungal polypeptide or is derived from a fungal polypeptide.

15

20

25

...

- 48. The isolated or purified polypeptide according to Claim 48, wherein said polypeptide is a  $\Delta 6$  desaturase.
- 49. An isolated nucleic acid encoding a polypeptide according to Claim 5 47 or Claim 49.
  - 50. The nucleic acid construct according to Claim 23, wherein said portion of an amino acid sequence depicted in SEQ.ID. NO: 2 comprises amino acids 1 through 457.
    - 51. A host cell comprising:

a nucleic acid construct according to any one of Claims 22 to 24.

52. A host cell comprising:

a vector which includes a nucleic acid which encodes a fatty acid desaturase derived from *Mortierella alpina*, wherein said desaturase has an amino acid sequence represented by SEQ ID NO:2, and wherein said nucleotide sequence is operably linked to a promoter.

- 53. The host cell according to Claim 52, wherein said host cell is a eukaryotic cell.
- 54. The host cell according to Claim 53, wherein said eukaryotic cell is selected from the group consisting of a mammalian cell, a plant cell, an insect cell, a fungal cell, an avian cell and an algal cell.
- 55. The host cell according to Claim 54 wherein said host cell is a fungal cell.

PCT/US98/07126 WO 98/46763

The host cell of Claim 21, wherein said promoter is exogenously 56. supplied to said host cell.

5

A method for production of stearidonic acid in a eukaryotic cell 57. culture, said method comprising:

growing a eukaryotic cell culture having a plurality of recombinant

eukaryotic cells, wherein said recombinant eukaryotic cells or ancestors of said recombinant eukaryotic cells were transformed with a vector comprising fungal

DNA encoding a polypeptide which converts α-linolenic acid to stearidonic acid,

functional in said recombinant eukaryotic cells, under conditions whereby said DNA

58. A method for production of linoleic acid in a eukaryotic cell

wherein said DNA is operably associated with an expression control sequence

is expressed, whereby stearidonic acid is produced from α-linolenic acid in said

10

eukaryotic cell culture.

culture, said method comprising:

15

Live are given given are the first than the given given are the first than the fi

20

25

eukaruyotic cells, wherein said recombinant eukaryotic cells or ancestors of said recombinant eukaryotic cells were transformed with a vector comprising fungal DNA encoding a polypeptide which converts oleic acid to linoleic acid, wherein said DNA is operably associated with an expression control sequence functional in said recombinant eukaryotic cells, under conditions whereby said DNA is expressed, whereby linoleic acid is produced from oleic acid in said eukaryotic cell culture.

growing a eukaryotic cell culture having a plurality of recombinant

**59.** . The method according to Claim \$7 or Claim 58, wherein said eukaryotic cells are selected from the group consisting of mammalian cells, plant cells, insect cells, fungal cells, avian cells and algal cells.

THE P. LEWIS

15

20

25

(O

j

1.

10

60. The method according to Claim 59, wherein said fungal cells are yeast cells of the genus Saccharomyces.

#### 61. A recombinant yeast cell comprising:

(1) at least one nucleic acid construct according to Claim 23 or 24; or

(2) at least one nucleic acid construct according to Claim 23 and at least one nucleic acid construct according to Claim 24.

### 62. A recombinant yeast cell comprising:

at least one nucleic acid construct comprising a nucleotide sequence which encodes a functionally active  $\Delta 6$  desaturase having an amino acid sequence which corresponds to or is complementary to all or a portion of an amino acid sequence depicted in SEQ ID NO: 2, and at least one nucleic acid construct comprising a nucleotide sequence which encodes a functionally active  $\Delta 12$  desaturase having an amino acid sequence which corresponds to or is complementary to all or a portion of an amino acid sequence depicted in SEQID NO: 4, wherein said nucleic acid constructs are operably associated with transcription control sequences functional in a yeast cell.

# 63. A method of making GLA, said method comprising:

growing a recombinant yeast cell according to Claim 62 under conditions whereby said nucleotide sequences are expressed, whereby GLA is produced in said yeast cell.

## 64. A method of making GLA, said method comprising:

growing a recombinant yeast cell according to Claim 61 under conditions whereby the nucleotide sequences in said nucleic acid constructs are expressed, whereby GLA is produced in said yeast cell.

WO 98/46763 PCT/US98/07126

65. A method for obtaining altered long chain polyunsaturated fatty acid biosynthesis comprising the steps of:

growing a plant having cells which contain one or more transgenes, derived from a fungus or algae, which encodes a transgene expression product which desaturates a fatty acid molecule at a carbon selected from the group consisting of carbon 6 and carbon 12 from the carboxyl end of said fatty acid molecule, wherein said one or more transgenes is operably associated with an expression control sequence, under conditions whereby said one or more transgenes is expressed, whereby long chain polyunsaturated fatty acid biosynthesis in said cells is altered.

66. The method according to claim 65, wherein said long chain polyunsaturated fatty acid is selected from the group consisting of 18:1ω9, LA, GLA, SDA and ALA.

67. A microbial oil or fraction thereof produced according to the method of claim 65.

68. A method of treating or preventing malnutrition comprising administering said microbial oil of claim 67 to a patient in need of said treatment or prevention in an amount sufficient to effect said treatment or prevention.

69. A pharmaceutical composition comprising said microbial oil or fraction of claim 67 and a pharmaceutically acceptable carrier.

- 70. The pharmaceutical composition of claim 69, wherein said pharmaceutical composition is in the form of a solid or a liquid.
- 71. The pharmaceutical composition of claim 70, wherein said pharmaceutical composition is in a capsule or tablet form.

Cultal

10

THE TO THE

Ιđ

5

15

20

25

WO 98/46763 PCT/US98/07126

The pharmaceutical composition of claim 69 further comprising at 72. least one nutrient selected from the group consisting of a vitamin, a mineral, a carbohydrate, a sugar, an amino acid, a free fatty acid, a phospholipid, an antioxidant, and a phenolic compound.

5

A nutritional formula comprising said microbial oil or fraction 73. thereof of claim 67.

74. The nutritional formula of claim 73, wherein said nutritional formula is selected from the group consisting of an infant formula, a dietary supplement, and a dietary substitute.

- *75*. The nutritional formula of claim 74, wherein said infant formula, dietary supplement or dietary supplement is in the form of a liquid or a solid.
- 76. An infant formula comprising said microbial oil or fraction thereof of claim 67.

20

ŧ. 🗓 ŧ.Ī

> 77. The infant formula of claim 76 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

25

The infant formula of claim 77 further comprising at least one **78**. vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

PCT/US98/07126 WO 98/46763

79. A dietary supplement comprising said microbial oil or fraction thereof of claim 67.

5

80. The dietary supplement of claim 79 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

10

81. The dietary supplement of claim 80 further comprising at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

15

82. The dietary supplement of claim 79 or claim 81, wherein said dietary supplement is administered to a human or an animal.

20

83. A dietary substitute comprising said microbial oil or fraction thereof of claim 67.

84. The dietary substitute of claim 83 further comprising at least one macronutrient selected from the group consisting of coconut oil, soy oil, canola oil, mono- and diglycerides, glucose, edible lactose, electrodialysed whey, electrodialysed skim milk, milk whey, soy protein, and other protein hydrolysates.

25

85. The dietary substitute of claim 84 further comprising at least one vitamin selected from the group consisting of Vitamins A, C, D, E, and B complex; and at least one mineral selected from the group consisting of calcium, magnesium, zinc, manganese, sodium, potassium, phosphorus, copper, chloride, iodine, selenium, and iron.

The dietary substitute of claim 83 or claim 85, wherein said dietary 86. substitute is administered to human or animal.

Last for the time of the

A method of treating a patient having a condition caused by 87. insuffient intake or production of polyunsaturated fatty acids comprising administering to said patient said dietary substitute of claim 83 or said dietary supplement of claim 79 in an amount sufficient to effect said treatment.

15

- The method of claim 87, wherein said dietary substitute or said 88. dietary supplement is administered enterally or parenterally.
- 89. A cosmetic comprising said microbial oil or fraction thereof of claim **67**.
  - The cosmetic of claim 88, wherein said cosmetic is applied topically. 90.

20

- The pharmaceutical composition of claim 69, wherein said 91. pharmaceutical composition is administered to a human or an animal.
- An animal feed comprising said microbial oil or fraction thereof of 92. claim 67.

25

The method of claim 20 wherein said fungus is Mortierella species.

and the state of good a vice factor becaute and a second

i) at least one mineral selected from the givent constituing of calcium, magnetum.



17 C. C.

Ų

THE PARTY OF THE PARTY OF THE

15

94. The method of claim 93 wherein said fungus is Mortierella alpina.

95. An isolated peptide sequence selected from the group consisting of SEQ ID NO:34 - SEQ ID NO:40.

96. An isolated peptide sequence selected from the group consisting of SEQ ID NO:20, SEQ ID NO:22, SEQ ID NO:25 and SEQ ID NO:26.

97. A method for production of gamma-linolenic acid in a eukaryotic cell culture, said method comprising:

growing a eukaryotic cell culture having a plurality of recombinant eukaryotic cells, wherein said recombinant eukaryotic cells or ancestors of said recombinant eukaryotic cells were transformed with a vector comprising fungal DNA encoding a polypeptide which converts linoleic acid to gamma-linolenic acid, wherein said DNA is operably associated with an expression control sequence functional in said recombinant eukaryotic cells, under conditions whereby said DNA is expressed, whereby gamma-linolenic acid is produced from linoleic acid in said eukaryotic cell culture.

20 17.6

98. The method according to Claim 97 wherein said eukaryotic cells are selected from the group consisting of mammalian cells, plant cells, insect cells, fungal cells, avian cells and algal cells.